

Human immunodeficiency virus infection of monocytes: relationship to Fc-gamma receptors and antibody-dependent viral enhancement

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SUMMARY

Antibodies that augment human immunodeficiency virus (HIV) infectivity of monocytes through Fc receptor (FcR) type III for IgG have been found in the blood of sero-positive patients and immunized chimpanzees. This study investigated the effect of acute and chronic HIV infection, as well as protein kinase C activators capable of up-regulating latent HIV, on the expression of these receptors. In addition, the frequency of this antibody-dependent enhancement (ADE) phenomenon was estimated using purified IgG from HIV-1 seropositive individuals at various clinical stages of infection. The existence of an FcR-dependent pathway for ADE of HIV-1 infection in peripheral blood monocytes and promonocytic U937 cells was confirmed in sera from a small subset of patients, and the phenomenon extended to FcR types I and II. The level of ADE activity was minimal, however, and no relationship between the presence or magnitude of the ADE phenomenon and clinical stage was uncovered. Finally, perturbations which activate a latent HIV infection were shown to concomitantly up-regulate FcR on infected and uninfected cells. This suggests a positive feedback loop linking up-regulation of latent infection, enhanced expression of low affinity HIV receptors such as FcR, and viral spread.

INTRODUCTION

Antibody-dependent enhancement (ADE) of viral infection is a phenomenon by which immunoglobulins facilitate uptake of a virus–antibody immune complex via Fc or complement (CR3) receptors on target cells (Porterfield, 1986; Bolognesi, 1989). Expression of alpha, pox, bunya, reo and herpes viruses may be augmented *in vitro* by 4 log-fold or greater following exposure to low affinity specific antibody (Porterfield, 1986; Bolognesi, 1989). Lesser degrees of enhancement have been reported using murine retroviruses (Legrain, Goud & Buttin, 1986). In addition, ADE has been loosely correlated to exacerbation of clinical disease (Porterfield, 1986; Halstead, 1988).

ADE has also been demonstrated with human immunodeficiency viruses (HIV) types 1 and 2 and simian immunodeficiency virus *in vitro* (Robinson *et al.*, 1989a, b). It raises the possibility that active vaccination or passive immunotherapy might result in similar augmentation of the cellular uptake and dissemination of HIV *in vivo*. While clinically apparent ADE has not been observed in short-term trials of various vaccines in HIV-infected individuals, suggestions of its occurrence in simian models (Prince *et al.*, 1988) are troublesome.

There is wide disparity in the magnitude and frequency with which ADE for HIV-1 and 2 is observed *in vitro*. These

differences may relate to the target cell used, its basal level of Fc receptor (FcR) and CR3 expression, the relative ratio of neutralizing to enhancing antibodies in a particular preparation, as well as the role of complement. In addition, some groups report the ability of anti-CD4 antibodies or soluble recombinant CD4 to block ADE (Jouault *et al.*, 1989; Matsuda *et al.*, 1989), a result denied by others (Homsy *et al.*, 1989). The present experiments were designed to investigate three basic issues related to HIV-1-specific ADE and FcR. The effect of HIV infection, and agents which can convert a chronic infection to a state of active viral replication, on the expression of promonocyte Fc receptors is first delineated. Second, the ability of purified IgG from HIV seropositive individuals to alter HIV infection of peripheral blood monocytes, and immortalized promonocytic cells with divergent levels of FcR, is described. Third, the possibility that ADE is related to clinical stage of HIV infection is explored.

MATERIALS AND METHODS

Cells

U1.1A cells were subcloned, by limiting dilution, from U1, a clone of U937.3 promonocytic cells infected with the lymphadenopathy-associated virus (LAV-1) strain of HIV-1 and obtained from T. M. Folks of the NIH, Bethesda, MD (Folks *et al.*, 1988). Uninfected U937 cells were plated at 0.3 cells per microwell and growth-positive wells screened for subsets differ-

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ing in levels of FcR type I (FcRI). Peripheral blood mononuclear cells were isolated from heparinized venous blood of HIV sero-negative donors by Ficoll-Hypaque (Pharmacia, Piscataway, NJ) density gradient centrifugation and monocytes prepared by plastic adherence, as described previously (Laurence, Gottlieb & Kunkel, 1983). Adherent cells ('monocytes') were 60% esterase⁺ and 85–90% CD11b (Mo1)⁺. All cells were cultured in RPMI-1640 (Flow Laboratories, McLean, VA) plus 10% fetal bovine serum (FBS).

HIV stock

The CD4⁺ lymphoblastoid cell line H9 was infected with a strain of HIV-1 known as HIV_B and used as a source for continuous production of virus. This isolate is highly T-cell trophic, yet capable of infecting cells of the monocyte/macrophage lineage (Gartner *et al.*, 1986). Supernatants were centrifuged at 2500 *g*, filtered through 0.22 μ membranes (Millipore/Continental Water Systems, Bedford, MA), and stored at -70° . Infectivity assays (Laurence *et al.*, 1989a) indicated that 1.0 ml of HIV_B stock corresponded to an ID₅₀ of 1000.

HIV infection of U937 clones

Cells were suspended at 0.5×10^6 /ml in culture medium, plated in macrowells, and exposed to viral stock for 2 hr at 37° , followed by washing with phosphate-buffered saline (PBS) and replating. One-half of the supernatant was removed and replaced with fresh medium every 3–4 days thereafter. Infection was confirmed and quantified by indirect immunofluorescence (IFA) using polyclonal human anti-HIV IgG (Laurence, Saunders & Kulkosky, 1987) and FITC-conjugated F(ab')₂ fragments of goat anti-human IgG, IgA and IgM (Cappel Laboratories, Cochranville, PA). Percentage infected cells and fluorescence intensity were determined by cytofluorimetry using an EPICS-V cell sorter.

Fc receptor expression

Fc receptor-gamma (FcR) expression was analysed by two methods, IFA (Anderson *et al.*, 1986) and binding of antibody-sensitized erythrocytes (EA) (Salmon *et al.*, 1990). IFA employed: mouse IgG2a (Tago, Burlingame, CA) binding to quantify FcRI; monoclonal antibody (mAb) IV.3 for FcRII (Anderson *et al.*, 1986); and mAb 3G8 for FcRIII (Fleit, Wright & Unkless, 1982). FITC-conjugated, affinity-purified rabbit anti-mouse IgG antibodies (Tago) served as the developing reagent. 1×10^6 viable cells were washed twice with PBS supplemented with 1% bovine serum albumen (BSA) and 0.1% sodium azide, pelleted, and incubated with 20 μ l of 20 μ g/ml IgG2a for 30 min on ice. After washing twice with PBS, the cells were incubated with 20 μ l of 0.1 mg/ml FITC-conjugated rabbit anti-mouse IgG antibody for 30 min on ice, washed, and analysed by flow cytometry.

Generalized FcR expression was analysed by EA binding. Bovine erythrocytes (Department of Veterinary Medicine, Cornell University, Ithaca, NY) were incubated with rabbit IgG anti-bovine erythrocyte antibody (Cappel Laboratories) for 1 hr at 37° . The amount of antibody used was a fourfold dilution of the minimal agglutinating titre. The cells were then washed, resuspended at 1×10^8 cells/ml in RPMI-1640 and 20% FBS, and 100 μ l added to 4-ml tubes containing 100 μ l of test cell populations at a concentration of 5×10^6 /ml. Tubes were centrifuged at 44 *g* for 3 min, kept at 25° for 20 min, then gently

Table 1. Expression of FcR on U937 cell clones*

Reagent	Specificity	Frequency of positive cells (%)			
		U937-11/19	U937-BK	U937.3	U1.1A
EA rosette	FcR	18	82	46	52
mAb anti-IgG2a	FcRI	<2	89	40	45
mAb IV.3	FcRII	16	60	34	ND
mAb 3G8	FcRIII	<2	<2	<2	<2

*Clones of U937 cells, either uninfected (11/19, BK, U937.3) or chronically infected with HIV-1 (U1.1A), were analysed for FcR expression using indirect immunofluorescence and flow cytometry (IFA), or rosetting with antibody-coated bovine erythrocytes (EA). U937.3 is the uninfected counterpart of U1.1A. Background immunofluorescence for mAb was $\leq 2\%$. Percentages represent the mean of two experiments.

ND, not done

resuspended. Toluidine blue, 20 μ l of a 0.2% solution, was added and EA rosettes counted using a haemocytometer. A rosette is defined as ≥ 3 erythrocytes adherent to a mononuclear cell.

Viral assays

HIV antigens were quantified in detergent-solubilized culture supernatants by an ELISA-based assay for viral p24 core protein using human Ig directed against p24 epitopes (Abbott Labs, Chicago, IL), as described elsewhere (Laurence, Sellers & Sikder, 1989b). Reverse transcriptase determinations were performed on PEG 4000 (Sigma Chemical Co., St Louis, MO)-precipitated culture supernatants as detailed previously (Laurence *et al.*, 1987). IFA for HIV-specific protein expression is described above.

Immunoglobulin preparations

Sera were obtained from 16 individuals at various stages of HIV infection and IgG isolated by ammonium sulphate fractionation and Zeta-Chrom 60 ion-exchange chromatography disc (CUNO Inc., Meriden, CT) filtration (Laurence *et al.*, 1989a). Stocks were prepared as 1 mg/ml in PBS.

ADE assay

Two-hundred microlitres of viral stock were incubated with 200 μ l of purified IgG of varying concentrations (0.1–25 μ g) at 4° for 2 hr, then added to 2.5×10^5 U937 cells or 5×10^5 peripheral monocytes in 0.6 ml culture medium for 18 hr at 37° . Cells were washed, resuspended in 2 ml of fresh medium, and HIV production monitored by measurement of p24 core protein, IFA, or reverse transcriptase activity at 3–4 day intervals.

RESULTS

FcR expression on U937 clones

Three clonal populations of U937 were utilized: U937.3, U937-11/19, and U937-BK. 11/19 and BK were selected by limiting dilution of U937 for low and high expression, respectively, of FcRI. Both are 100% CD4⁺. The majority of cells in clones U937-BK and U937.3 exhibited FcRI and FcRII; all lacked FcRIII (Table 1). U937-11/19, selected for the absence of

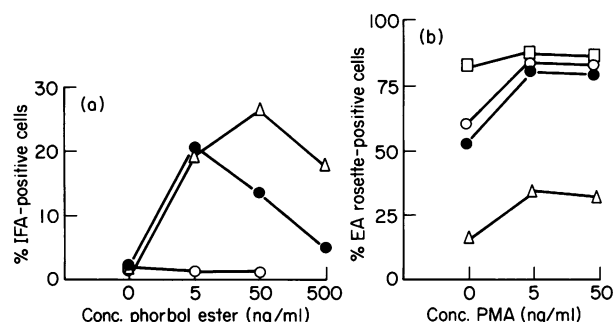


Figure 1. Effect of phorbol esters on FcR expression in U937 cell clones. (a) U937-11/19 cells were exposed to varying concentrations of three different phorbol esters (Δ , PMA; \bullet , PDB; \circ , 4B-phorbol). FcRI were evaluated at 48 hr by immunofluorescence assay for reactivity with FITC-mouse IgG. (b) Four different clones of U937, infected with HIV-1 (U1.1A, \bullet) and uninfected (\circ , U937.3; Δ , U937-11/19; \square , U937-BK) were exposed to varying concentrations of PMA. FcR were evaluated at 48 hr by EA rosette assay.

Table 2. Levels of HIV-1 antibody-dependent enhancement in IgG from individuals at varying clinical stage of HIV infection

Clinical stage	No. of individuals	Conc. IgG (μ g)	No. ADE positive	ADE level*
Asymptomatic	6	1	1	2.6
		20	0	—
Lymphadenopathy syndrome	6	1	4	4.2, 2.7, 4.2, 2.5
		20	0	—
AIDS†	4	1	0	—
		20	0	—
Control, HIV seronegative	5	1	—	0.98 \pm 0.10

* ADE level = (HIV activity_{experiment}) / HIV activity_{buffer}.

† All had opportunistic infections, or Kaposi's sarcoma plus such infections.

staining with IgG2a and FITC-rabbit anti-mouse reagents, did exhibit weak EA rosetting in a fraction (<20%) of cells (Table 1).

Effect of HIV infection on FcRI

No difference in EA rosetting or FcRI and -III expression between chronically infected U1.1A cells and the parental clone U937.3 was apparent (Table 1). Acute HIV infection similarly had no effect on U937-BK cells maximally expressing FcRI, while only minor changes (<10% increase in positive cells) could be noted in FcRI expression on the U937 11/19 clone.

Up-regulation of FcR expression by phorbol esters

PMA augments HIV replication in chronically infected U937 cells (Folks *et al.*, 1988), and also enhances FcR expression in its

uninfected counterpart (Harris *et al.*, 1985). As FcR also appear to facilitate HIV-1 entry into U937 cells (Takeda, Tuazon & Ennis, 1988), it was of interest to examine the effects of phorbol esters on FcR expression in actively and chronically infected U937 cells. FcR were measured after a 48-hr exposure of cells to PMA or its analogue PDB (4B-phorbol 12,13-dibutyrate), the optimum period as determined in preliminary kinetic experiments. A concentration-dependent up-regulation was noted by the IgG2a binding (Fig. 1a) and EA rosetting (Fig. 1b) methods in both uninfected and chronically infected clones. This was particularly striking in the clone with low baseline levels of FcRI expression (Fig. 1a). 4B-phorbol, a congener of phorbol myristate acetate (PMA) incapable of altering protein kinase C (PKC) or diacylglycerol levels (Laurence *et al.*, 1990), had no effect.

Parallel cultures of U1.1A cells were exposed to varying concentrations of PMA or PDB, and supernatants assayed for HIV p24 core antigen at 48 hr. As previously shown (Folks *et al.*, 1988; Laurence *et al.*, 1989a), a 5–25-fold increase in p24 antigen occurred in the presence of PMA or PDB. Baseline concentrations of p24 in the absence of stimulus was 2920 pg/10⁴ cells, rising to 32,440 \pm 4920 pg with 50 ng/ml PMA and to 59,400 \pm 6320 with 50 ng/ml PDB. 4B-phorbol had no effect (3600 pg at 50 ng/ml).

ADE in IgG preparations from HIV sero-positive individuals

Total IgG was isolated from sera of 16 individuals [13 homosexual males and three females who acquired HIV heterosexually (2) or through blood transfusion (1)], at various clinical stages of HIV infection. ADE activity was assessed by measurement of p24 levels in culture supernatants of U937-BK cells 8 days after exposure to HIV-IgG complexes (Table 2). Enhancement indices were calculated by comparison of viral activity to buffer. An ADE index > 1.3 was regarded as positive, as none of five control sera exceeded 95% confidence limits for this index (mean 0.98 \pm 0.10). The magnitude of these indices was identical to those obtained in a similar system by Takeda *et al.* (1988). No significant differences were seen between ADE levels of asymptomatic individuals versus those with HIV-related disease.

Comparison of ADE in clones of U937 with divergent expression of FcRI and -II

The possibility that the magnitude of the ADE phenomenon might be affected by levels of FcR on target cells was pursued using clones selected for high and low expression of FcRI and -II. Both clones had equivalent expression of CD4 in terms of frequency of positive cells (100%) and density per cell (fluorescence intensity of 110 on a log scale). As shown in Table 3, using an IgG with a particularly high level of ADE, the fact that FcRI differed by >40-fold and FcRII by almost fourfold on U937-BK versus U937-11/19 had little effect on the level ADE index. This was true whether ADE was assessed by the frequency of cells infected in a population (IFA assay) or by the amount of virus produced (p24 determinations). However, the dependence of ADE on FcR expression and function was demonstrated using heat-aggregated IgG (HAI) to block FcR. Twenty-five micrograms of HAI were able to partially (Patients-RS) or completely (Patient-Tub) suppress ADE (Table 4).

Table 3. Comparison of ADE in promonocytic clones selected for high (U937-BK) versus low (U937-11/19) expression of FcRI and II

Subject	Conc. IgG (μ g)	HIV Assay	Antibody-dependent enhancement			
			U937-BK		U937-11/19	
			HIV activity*	ADE level†	HIV activity	ADE level
None	—	p24 Ag	521 \pm 188	1	485 \pm 144	1
Control-SJ	2	p24 Ag	546	1.0	550	1.1
Patient-Sec	2	p24 Ag	1099 \pm 71	2.1	2055	4.2
None	—	IFA	1.21 \pm 0.48	1	3.43 \pm 0.74	1
Control-JL	2	IFA	0.99	0.8	2.98	0.9
Patient-Sec	2	IFA	8.09	6.7	23.0	6.7

*HIV activity determined on Day 8 following exposure of 2.5×10^5 cells to HIV-IgG complexes. Indirect immunofluorescence (IFA) determinations are expressed as percentage specific staining with a human polyclonal anti-HIV IgG. p24 antigen determinations are expressed as pg of HIV core protein.

†Determined as described in the legend to Table 2.

Table 4. Effect of FcR blockade by heat-aggregated IgG on antibody-dependent viral enhancement in U937 cells

Subject	Conc. IgG (μ g)	HIV activity		
		HAI*	% IFA	ADE level
None	—	—	28.2 \pm 3.0	1
Patients-RS	2	—	75.5 \pm 4.1	2.7
	20	—	16.5 \pm 5.1	0.6
Patients-RS	2	+	40.9 \pm 5.7	1.5
	20	+	2.6	0.09
Patient-Tub	2	—	69.9 \pm 1.0	2.5
	20	—	77.1 \pm 11.3	2.7
Patient-Tub	2	+	ND	—
	20	+	29.0	1.0

*25 μ g heat-aggregated IgG (HAI), prepared from an HIV seronegative donor, were added to U937-BK cells for 2 hr, followed by exposure of cells to IgG-HIV complexes. HIV activity was assessed by indirect immunofluorescence (IFA) on Day 10 following infection.

Comparison of ADE on immortalized promonocytic cells versus peripheral monocytes

The possibility that ADE levels, as commonly determined on U937 lines, might not be applicable to non-transformed, non-replicating peripheral monocytes was pursued by direct comparisons. Concentrations of IgG capable of augmenting HIV expression did so on both types of cell, albeit with greater magnitude for the cell line (Table 5). Similarly, concentrations of IgG capable of neutralizing HIV did so on both cell types.

ADE and optimization of HIV infection

The previous experiments all utilized a fairly high dose of HIV (200 TCID₅₀, or 50% tissue culture infectious doses). The

Table 5. Comparison of antibody-mediated enhancement or suppression of HIV infection in promonocytic cells versus peripheral blood monocytes

Subject	Conc. IgG (μ g)	HIV activity*			
		Peripheral monocyte		U937-BK	
		% IFA	ADE level†	% IFA	ADE level
None	—	4.4	1	2.3	1
Control-MB	2	3.6	0.8	2.2	1.0
	20	3.7	0.8	2.0	1.0
Patient-Sec	0.5	15.1	3.4	23.3	10.1
	2	13.7	3.1	14.0	N
	20	1.5	0.3	0.8	0.3
Patient-RS	0.5	4.4	1	1.9	0.8
	2	9.6	2.2	15.2	6.6
	20	0.2	0.05	0.4	0.2

*HIV activity was determined by indirect immunofluorescence (IFA) on Day 11 (peripheral blood adherent cells) or Day 8 (U937) following exposure of target cells to HIV-IgG complexes.

†Determined as described in the legend to Table 2.

possibility that IgG with ADE activity might be able to augment infection using lower, more physiological viral concentrations was explored. As shown in Table 6, infection of U937 cells with 10 TCID₅₀ resulted in particulate reverse transcriptase activity of only three times background levels on Day 7. This was increased slightly by the addition of an anionic resin (polybrene), but elevated to optimal levels by exposure to an ADE IgG.

DISCUSSION

HIV penetrates cells via a pH-independent membrane fusion event (Stein *et al.*, 1987). This is directed by its bipartite envelope glycoprotein, gp120, non-covalently linked via amino terminus

Table 6. Effect of enhancing antibody on optimization of HIV infection*

HIV TCID ₅₀	Preincubation	Reverse transcriptase activity (c.p.m.)
500	Buffer	24,106 ± 39
10	Buffer	9614 ± 201
500	Control-JL	28,845
10	Control-JL	7700
500	Polybrene	27,993
10	Polybrene	13,166
500	Patient-Cul	29,801
10	Patient-Cul	32,495 ± 1786

* U937-BK cells were exposed to buffer or polybrene (2 µg/ml) in the presence of two concentrations of viral stock alone (buffer) or in the form of HIV-IgG complexes. Control and patient sera were used at 20 µg. Reverse transcriptase activity was assessed on culture supernatants 7 days following viral inoculation. Background reverse transcriptase activity was 3092 c.p.m.

interactions to a transmembrane domain, gp41, imbedded in the lipid membrane that surrounds the virion (Gelderblom *et al.*, 1987). The functional envelope ligand appears to be a multimer of this basic structure, with multi-unit interactions probably accounting for the high K_d of receptor-CD4 association, to the order of 10⁻⁹ M. However, alternate, low affinity receptors also appear to exist, enabling uptake of HIV into cells which lack detectable surface CD4 or CD4 transcripts. These include such diverse cells as neurons (Harouse *et al.*, 1989), endothelial cells (Teitel *et al.*, 1989) and fibroblasts (Tateno, Gonzalez-Scarano & Levy, 1989).

Fc receptors appear to be involved in certain of these low-affinity HIV interactions. By analogy with other enveloped viruses, it is probable that low affinity antibodies form monovalent complexes with FcR and HIV and facilitate viral entry through a pinocyte-endosomal pathway (Bernard *et al.*, 1990). This study has focused on FcR-gamma and its relationship to ADE. It is shown for the first time that the same activation signal, phorbol ester exposure with presumptive up-regulation of PKC activity, augments both HIV replication and FcRI and II expression in chronically infected cells. Also, the persistence of both types of receptor on chronically and acutely infected promonocytic cells has been demonstrated. These latter results parallel studies of FcRII expression on acutely infected U937 cells (Petit *et al.*, 1988), and conform with the normal or increased levels of FcRI and FcRII on peripheral monocytes from AIDS patients (Davidson *et al.*, 1988).

Subsequently, a complement-independent ADE phenomenon has been identified in a minority of sera from HIV-infected individuals but, at least in the small sample studied, a correlation with clinical status could not be detected. This ADE was FcR dependent, being blocked by preincubation of cells with heat-aggregated IgG. These data confirm the existence of an FcR-dependent ADE phenomenon, and emphasize its low level of activity.

The slight enhancement of infectivity observed with most of

the IgG, with few samples giving an ADE index > 2.5, is similar to findings of some groups using a variety of assay systems (Jouault *et al.*, 1989; Takeda *et al.*, 1988), yet much lower than reported by others (Homsy *et al.*, 1989; Robinson *et al.*, 1989b). The reasons for this are unclear. It is unrelated to the presence of complement as, in a pilot study, addition of fresh serum had little effect on ADE levels of selected IgG (data not shown). The possibility that ADE levels may differ depending on the molecular clone of HIV-1 or HIV-2 examined requires further attention, as suggested by the work of Homsey *et al.* (1989). Indeed, ADE may directly parallel clinical progression when sera are paired with homotypic HIV isolates (Homsy, Meyer & Levy, 1990).

Further work is also required to sort out the significance of the divergent types of ADE described by various groups. For example, ADE specific for HIV was first noted as a factor present in 3 of 34 (8.8%) serum samples from HIV seropositive individuals capable of augmenting infectivity of MT-2 CD4⁺ T lymphoblastoid cells (Robinson, Montefiori & Mitchell, 1987). Shortly thereafter, IgG-mediated ADE for HIV in human monocytes and U937 cells, but not T or B lymphoblastoid cell lines, was described (Wigzell, 1988). Since that time controversy has surrounded the incidence and magnitude of the ADE phenomenon, as well as its dependence on FcR, CD4 and complement.

It has been suggested (Robinson *et al.*, 1989b) that two types of ADE exist in AIDS: complement-mediated as well as complement-independent, FcR-mediated processes. This classification is inadequate, however, as the complement-independent process has alternately been described as an adjunct to CD4-mediated viral entry (Jouault *et al.*, 1989; Matsuda *et al.*, 1989), or independent of CD4 (Homsy *et al.*, 1989). In addition, the type(s) of FcR involved is unclear.

Three distinct classes of human leucocyte FcR have been identified by functional criteria and reactivity with mAb (Hogg, 1988). The high-affinity FcR, FcRI, is only present on mononuclear phagocytes, including U937. Monoclonal anti-FcRIII, but not anti-FcRI or FcRII, blocked ADE in peripheral blood macrophages (Homsy *et al.*, 1989), yet ADE has been recorded in U937 cells in the present work, and by others (Takeda *et al.*, 1988; Matsuda *et al.*, 1989), even though U937 lack FcRIII.

The biological significance of ADE must await data from ongoing clinical trials of active vaccination and passive immunization in AIDS. However, there are several other implications to the present work. As PKC-dependent signals such as PMA enhanced FcR expression on cells of monocytic lineage, stimuli such as lymphokine exposure or antigen stimulation which up-regulate HIV replication through similar PKC-related pathways may also facilitate spread of virus, via increased FcR, to other cells. In addition, other viruses which frequently infect HIV seropositive individuals, including cytomegalovirus, Epstein-Barr virus and herpes simplex virus, can induce FcR on endothelial and other cells (Johansson *et al.*, 1989), which are secondary targets for HIV, and this should be considered as one means by which these agents serve as co-factors in HIV disease.

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